Vanadium.

λ.	Int. in spark.	Int. in arc.	Remarks.
3827·30	2—3	trace	
3867·00	3	2	
3878·90	8	4	
3885·05	3	2	
3899·30	6	3	
3903·40	6	4	
3914·44	6	4	
3916·50	6	4	
3952·07	7	5	
3973·85	5	4	
3985·90	1	0	
3997·30	5	4	
3999·30	2	0	
4005·85	10	7	
4017·00 } 4017·40 }	1	0	
4023·60	9	7	
4035·80	8	6	
4053·80	2—3	trace	Enhanced Ti line at λ 4053·98.
4061·80	2—3	1	
4065·20	3—4	0	
4178·50	1—2	1	
4183·60	4	3	
4202·55	3	2—3	
4205·24	4	3	
4225·41	3	1—2	
4232·20	1—2	0	
4243·10	1	0	

"The Colour-Physiology of *Hippolyte varians*." By F. W. KEEBLE, Caius College, Cambridge, and F. W. GAMBLE, Owens College, Manchester. Communicated by Professor S. J. HICKSON, F.R.S. Received October 25,—Read November 23, 1899.

The following paper gives in a categorical fashion the chief results of a research on the changes of colour in the prawn *Hippolyte varians*. The work was carried out last year partly in the Zoological Laboratories of Owens College, Manchester, partly at the station furnished by the Lancashire Sea Fisheries Committee at Barrow; and during the past summer in M. Perrier's Laboratory at St. Vaast, Normandy. A fuller description of the experiments, together with figures, will appear shortly. The present abstract contains the following sections:—

I. Previous knowledge of colour-change in *Hippolyte varians*.
II. Methods adopted for obtaining reliable colour-records—
  a. Colour registration.
  b. Chromatophore examination.

III. The nature of the "chromatophores" and their pigments.
IV. The habits of *Hippolyte varians*. Sexual dimorphism.
V. The nocturnal colour. Nocturnes.
VI. Periodicity of colour-change.
VII. Range of colour-change.
VIII. The causes of change in colour.
 a. Colour of the surroundings.
 b. Light-intensity.
 c. Electric and other stimuli.
IX. The rôle of the eye and nervous system in the control of the colour-form.
X. The "chromatophores" of larval forms.

I. *Previous Knowledge.*

The facts previously known may be arranged in three groups. The great variety of colour displayed by different specimens of *Hippolyte varians*; the "mimetic resemblances" between these colour-forms and the Algæ upon which they live; and the power which these colour-forms possess of undergoing a change of tint under different conditions of illumination. It is known that the so-called "chromatophores" contain differently coloured pigments by which both the prevalent tint of the prawn and its colour-changes are determined. The arrangement of these colour-elements in the body and the pigmentary conditions of the various colour-varieties have not been hitherto carefully examined. The factors which determine a change of colour, the extent of these changes, and the mode in which they are effected, may be said to be hitherto quite unknown.

II. *Method of Obtaining Reliable Colour-records.*

(*a*) Records of colour must be made under constant conditions of illumination, otherwise they are not strictly comparable, and fine shades of colour escape notice. They must be made rapidly, otherwise the light used for recording induces a colour-change and becomes, instead of a guide, a source of error. Finally, the light must be such as to enable a speedy record to be made, and yet one which itself induces a minimum change.

Many devices have been tried, but no completely satisfactory method has been obtained. We use, as most convenient, bright diffuse sunlight reflected from a white ground; and for a comparison of day and night colours, incandescent light. There are, however, several objections to these modes of illumination, the chief among them being that under certain circumstances, white light produces a very rapid change of colour.

(*b*) During microscopic examination, grave colour-changes often occur; yet with practice, a very rapid examination may be made, and so the source of error considerably reduced. The colours to be recorded are often several, the gross colour of the animal being seen under the microscope to be due to several pigments (see below Sect. III). These pigments are differently distributed in different colour-forms, so that the pigmentary records become rather complicated. Any but the briefest microscopical examination throws the nervous system of the animal out of gear, produces after-effects, and too frequently renders the animal useless for further experiment. Control-specimens must be used before conclusions can be drawn from the simplest experiments, and experiments must be confirmed several times. Added to these difficulties is this, that colour-change in *Hippolyte* is no simple reflex affair taking place "with the certainty of a physical experiment," but is one subject to what, in times of difficulty, seems to amount to wilful perversity. What we believe to be the chief element in this seeming perversity is described under the head of Periodicity in Section VI.

III. *The Nature of the "Chromatophores."*

The colours of the pigments in the "chromatophores" determine the tint of the prawn by their disposition and the depth of its colour by their abundance. The "chromatophores" are by no means the simple, stellate, cellular, dermal structures which they are commonly supposed to be. One series of them lies under the epidermis, another is interspersed between the muscle-fibres both of the great flexors and extensors of the tail and those of the appendages, while a third series—often forming great splashes of colour—invests the gut, nerve-cord, liver, and other internal organs.

In simple colour varieties—brown for instance—the pigment of the skin forms a dense network obscuring the "muscle-chromatophores." In such cases the colour of the prawn is determined by the colour of the superficial network. In other cases, when the prawns are banded or boldly barred, the colour-elements of the skin are absent or have no pigment, and the deep "chromatophores" alone determine the colour of the pattern. In many *Hippolyte* we have found that distribution of the pigment is the same in the skin-chromatophores and in the muscle-fibres which underlie them. The two sets are co-ordinated. This correspondence applies to other Crustacea, though it has not hitherto been recognised.

The pigments present in the chromatophores are limited to red, red and yellow, or red, yellow, and blue. These three may be present together in one and the same element. During colour-changes they are distributed independently of one another in the sense that one pigment may become aggregated in the centre of the "chromatophore,"

whilst another runs out into its network of processes. Change of colour appears to be due to a fresh pigmentary deal of the shuffled colour-pack.

Whether chemical changes play a part in converting one pigment to another or no, we are not yet in a position to say. All the evidence we have is against the view that the colour-elements of *Hippolyte* are cells like the chromatophores of the frog. The "muscle-chromatophores" bear tubular processes limited by a distinct membrane. The processes of the skin-chromatophores penetrate between the epidermal cells and form networks. The movement of the pigment is not due to a change in the form of the "chromatophore" but to a movement flowing to or from the central part. Further details of these colour-elements are given in our larger paper.

IV. *The Habits of* Hippolyte varians.

Hippolyte lives in swarms amongst the weeds of the seashore. In some places it is most abundant upon the *Halidrys* and other algæ which flourish luxuriantly in the "laminarian" zone, and are only exposed by very low spring-tides. In other places the *Zostera*-beds and the masses of *Fucus* form its chief resorts. Each colour-variety is a marvel of protective coloration. Each is to be found among weeds of a closely similar hue and adheres to its chosen habitat with the greatest tenacity. Though it has the power of making powerful leaps and of swimming, only under the greatest provocation can *Hippolyte* be induced to take this exercise. At night as well as during the day these prawns are still to be found on their food-plants; and, should the receding tide lay the weeds bare, the *Hippolyte* may still be found by shaking them into a net. Should the special food-plants of any given colour-varieties be mixed with other weeds, the prawns will after a time select, each after his kind, the weeds in which it naturally feeds and with which it agrees in colour. Generally speaking, *Hippolyte* prefers shade to direct sunlight or to artificial light. The emerald green variety found on *Zostera*, whether at a comparatively high zone on the shore or in the "laminarian" zone, is exposed to a considerable amount of light on account of the "blades" of this grass being separated from each other and not growing in the shade of deep rock pools. The brown and red varieties of *Hippolyte* are, on the other hand, associated with dense masses of weed attached to rocks; so that the light-intensity in which they live, even at half-tide, is probably lower than that of the beds of *Zostera*. The bearing of these facts on the changes of colour are referred to in Section VIII.

Hippolyte exhibits a certain sexual dimorphism both with regard to size and to colour. This may be expressed by saying that the males are on the average much smaller and less elaborately patterned than

the females, which are more resourceful in adjusting their colour to their surroundings. From the point of view of "protection" this is what might be expected owing to the greater sluggishness of the female, which in turn is partly due to the large mass of eggs or developing zoeæ, which she almost invariably carries.

V. *The Nocturnal Colour.* "*Nocturnes.*"

Whatever the diurnal colour of *Hippolyte* may be, it changes at or soon after night-fall to a wonderfully beautiful transparent blue or greenish-blue colour. Prawns in this condition we designate as Nocturnes. The depth of the nocturnal tint is directly proportioned to that of the diurnal colour; dark brown prawns becoming deep blue and light ones pale blue. Under natural conditions the nocturnal colour persists until daybreak. At the first touch of dawn the colour vanishes and that of the preceding day re-appears. Specimens trawled at night and in the early morning before daylight show that the nocturnal colour is perfectly normal and is assumed by *Hippolyte varians* while still on its food plant. Other Crustacea too, show a peculiar nocturnal colour. *Mysis*, for example, of different species and possibly even of distinct genera, show a transparent and blue colour-phase at night, giving place during the day to a deeply pigmented condition.

Nocturnes are remarkable chiefly but not solely for their peculiar pigmentary condition. The red and yellow pigments are maximally contracted, while the blue is present in a very diffuse homogeneous condition forming a network which traverses the connective tissue of all the chief organs, particularly the muscles. The peculiar transparency which accompanies this nocturnal condition is, however, not entirely explicable by the retraction of the red and yellow-coloured pigment. It is only one of a number of profound changes affecting the body as a whole. Indeed we are prepared to say that the nocturnal state opens up a new chapter in biological investigation, and that by a study of this condition increased knowledge of the succession of metabolic processes may be gained.

VI. *Periodicity of Colour-change.*

Under normal conditions *Hippolyte varians* passes through a daily colour-cycle. Its diurnal colour gives place to a slight increase of reddishness—a sunset-glow—just before night-fall, and this ushers in the nocturnal phase. These changes are periodic in the strict sense of the word. Though often modified by external agents they exhibit a certain independence of them. In constant darkness a Nocturne recovers its diurnal colour. In constant light (of certain kinds at

least) a diurnal form passes over to the nightly phase. Though light often induces and induces with marvellous rapidity a recovery from the nocturnal phase, it is often powerless to overcome the habit of the animal. The periodicity is only slowly worn down in the course of two or three days. These changes express a nervous rhythm; perhaps a profound and rhythmic course of metabolic events. The reddish phase antecedent to the full nocturnal tint probably explains the statement made by M. Malard,* that in darkness *Hippolyte varians* becomes red.

Periodicity is manifested in the colour-change of *Hippolyte* which have been deprived of both their eyes. The assumption of, and recovery from, the nocturnal phase is still effected, but more slowly and erratically than in normal specimens.

VII. *Range of Colour-change.*

Adult animals when placed with weed of a new colour (the light-intensity being as far as possible unaltered) are, under the conditions of the laboratory, only capable of very slow sympathetic colour-changes. Thus green *Hippolyte* placed on brown weed conserve their greenness for a week or more, but in the end give way and become brown. Their subsequent recovery when placed with green weed is more rapid. We have repeated such experiments in the open time after time, and have found that the prawns were either quite refractory or responded in this slow manner. Yet these same specimens undergo the changes preceding and culminating in the nocturnal colour and the succeeding recovery to their diurnal tint, with the utmost readiness. The fact that prawns, refractory to sympathetic colour-change can and do undergo a rapid change of tint when the light-intensity or the quality of the light is altered, is shown by such records as the following. A specimen, one of a large catch, incidentally observed to be the blackest we have ever seen, became in a few minutes transparent when put in a white porcelain dish. Further, a ready and almost infallible means of producing transparent green *Hippolyte* and even a colour hard to distinguish from the nocturnal tint, consists in placing freshly caught prawns in a white porcelain dish, and covering the top with a piece of muslin. Under these circumstances the change often takes place very rapidly (thirty seconds to one minute).

It is therefore necessary to distinguish at least three kinds of colour-change in *Hippolyte varians*. First, the passage from the diurnal to the nocturnal colour-phase followed by recovery to the colour of the previous day. In this case the phases form a rhythmic daily cycle. Second, the colour-changes produced by artificially altering the light-

* 'Bulletin de la Société Philomathique de Paris,' sér. 8, vol. 4, 1892, p. 28.

intensity to which the prawns are exposed, or by subjecting them to light reflected from white, and especially porcelain surfaces. Third, the sympathetic colour-change brought about by change in the colour of the surroundings.

The first of these is habitual or periodic, and may be quickly produced, towards evening, by a profound alteration of the light-intensity. Even the natural recovery from the nocturnal to the diurnal colour takes place rapidly with the dawn. The second change, as inexplicable in teleological terms as the first, is also rapid, often very rapid.

The third change is extremely slow. The prawn, in the acquirement of its adult colour, is guided and guided solely, so far as external circumstances are concerned, by light-intensity. In response to the conditions of light-intensity which prevail in its habitat, the prawn metabolises and distributes its pigments. But its pigmentary forces do not admit of ready mobilisation for purposes of defence; or at least they do not quickly obey a command to move. *Hippolyte* by its immobility has gradually grown into its surroundings and though, as for example, at night, its pigments may be readily aggregated, and a special nocturnal colour produced, yet this mobilising power is not utilised at all, or but very slowly, to redistribute the pigments, when the colour of the habitat is changed.

VIII. *The Causes of Change of Colour.*

(*a*) *Hippolyte* grows into harmony with its surroundings. So developed it hangs on to wave-swept weeds. Should it be dislodged its hope of concealment lies rather in a rapid choice of a weed of its own colour, than in a slow sympathetic colour-change on its own part, for if we may trust our experimental results, a week would elapse before the change could be complete. Monochromatic light (obtained by the use of Landolt's colour-filters), is singularly inefficacious in producing any sympathetic colour-change. Red, yellow, green, and blue light act in this respect like darkness. Under natural conditions we conclude, therefore, that the ultimate colour-change is effected by a reaction to light-intensity.

(*b*) After much trouble and many experiments, we find that there is no evidence that rays of light, by virtue of their specific wave-lengths, play any part in changing the colour of the prawns. On the other hand we find that in the diurnal phase, a low light-intensity favours expansion of the red pigment and so brown effects, while increased light-intensity produces a green tint. The appreciation of light-intensity appears to be very acute and to be the chief agent in producing colour-change.

(*c*) By ablation of the eyes, electrical stimuli, and heat, colour-changes may be induced. These agents have been employed in tracing

the nervous mechanism of the change, but the histological examination of these experiments is not yet complete.

IX. *The Rôle of the Eye and Nervous System.*

Removal of one eye produces no effect on the body-colour. Removal of both, either produces no effect or a rapid nocturning. The animal is plunged in night. Under such circumstances the periodic habit may re-assert itself and a recovery with subsequent fairly punctual nocturning take place. The chromatophores of detached limbs, and in the bodies of blinded specimens, exhibit alterations of their pigments—when subjected to different light-stimuli, quite similar to those which occur in the intact animal under the same conditions. Therefore (1) an intrinsic rhythmic nervous change supervises the periodic change in the pigments; (2) the eye is the most important auxiliary in modifying nervous control; (3) local government plays a part. In variegated colour-forms, which show, as in a mirror, the pattern of their weed, it can scarcely be doubted that both central and local government co-operate, and so produce a result of such consummate delicacy. Here there is expansion of one pigment, here of another, there complete contraction. The light acting through the eye on the central nervous system cannot be supposed to differentiate itself into such diverse stimuli as are required to produce the colour-variety. Local control under a strong central organisation seems to be the only likely force and the evidence favours this view.

X. "*Chromatophores*" *of larval forms.*

We have succeeded in hatching out the zoeæ of *Hippolyte varians* and in following their development for a short time. Several of the colour-elements acquire their pigment before the time of hatching. There are two pigments, one red and the other yellow by reflected and dull green by transmitted light. A few chromatophores contain red only. All the colour-elements are distributed symmetrically. They occur in the neighbourhood of the eyes, the liver, and at the sides of the abdominal segments.

In the zoea the pigments react with astonishing rapidity to certain changes of light-intensity. A bright light brings about very rapid contraction, while a dark background effects expansion of the yellow-green and more slowly a similar change in the red. A diffuse blue substance was noticed frequently as though exuding from the dense red body of some "chromatophores." This may be the result of a destructive action of light upon one or both of the pigments, and should this be so, we may find in a study of the larval stage the meaning of the blue colour of Nocturnes.